IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Lustig et al.	Group Art Unit: 1646
Serial No. 08/975,614	Examiner: Pak, M.
Filed: November 21, 1997	Attorney Docket No. T97-012
For: *Nuclear Hormone Receptor Drug Screens*	

DECLARATION UNDER RULE 132

I, Steven L. McKnight, declare and state as follows:

1. I am a Professor and Chairman of the Department of Biochemistry at the University of Texas Southwestern Medical Center. I am a cofounder and board member of Tularik Inc., the assignee of this application. I am a member of the National Academy of Science, an expert in the field of transcriptional activation and have authored numerous publications in this field.

2. Because nuclear hormone receptors provide uniquely easily targeted, specific handles for regulating gene expression and physiological function, they are routinely exploited as commercial drug targets without knowledge of their "natural" ligand(s), binding site(s) or particular function. These receptors without known natural ligand(s) are often called "orphan" receptors. In fact, orphan receptors provide some of the "hottest" present receptor targets in the pharmaceutical industry (see, e.g. Blumberg et al., 1998, Genes & Dev 12, 1269-77; Dussault et al., 2001, J Biol Chem 276, 33309-33312; Enmark and Gustafsson (1996) Mol Endocrinol 10:1293-1306).

3. Heery et al. (1997, Nature 387,733-36) describes three experiments: the first is an in vivo yeast-based two-hybrid experiment wherein a DNA-binding domain fusion protein comprising LXXLL motifs activated transcription through a ligand-binding domain of an estrogen receptor (Fig.1). The second experiment is a GST pull-down experiment wherein GST-ER fusion proteins pulled down in vitro translated ^{35}S-labeled natural-sequence SRC-1 proteins, but not otherwise identical mutant-sequence SRC-1 proteins wherein all four functional LXXLL motifs were disabled (Fig.3a). In this experiment, the natural sequence SRC-1 pull down was inhibited by uM concentrations of LXXLL peptides (Fig.3b). The third experiment showed that natural SRC-1 but not mutant SRC-1 increased

activation of estrogen receptor in HeLa cells transiently transfected with a reporter plasmid (Fig.3c).

One skilled in the art would not construe Heery to suggest the feasibility of assaying direct, in vitro LXXLL peptide binding to purified receptor; in fact, to one skilled in the art, Heery suggests the opposite - that such an assay would not be feasible. First, Heery provides no data suggesting an LXXLL peptide can directly bind the receptor. Heery's two-hybrid transcriptional activation is performed within yeast cells, the GST-pull down assay is performed in a crude cellular lysate, and the third, transient transfection experiment is cell-based. One skilled in the art would recognize that all of these experiments report both higher and lower order complex formation and that none of them implies direct peptide-receptor binding.

Similarly, none of Heery's data imply that an LXXLL peptide is sufficient to bind the receptor; in fact, they suggest the opposite. For example, one skilled in the art would recognize that Heery does not report any GST-pull down of an LXXLL peptide, nor any data wherein a single LXXLL motif was disrupted, but only wherein all four were disrupted. In fact, one skilled in the art would conclude that the author's failure to provide such data suggests that single LXXLL-motif disruptions may not have worked. In fact, this negative inference is further compelled by the subsequent inhibition experiments, wherein the authors only report data wherein uM concentrations of peptide were required to inhibit pull down - orders of magnitude higher than the amount of the SRC-1 protein present (the disclosed in vitro transcriptional yield is on the order of a few nM). One skilled in the art would conclude that the disproportionately high concentration of peptide necessary to inhibit SRC-1 pull down suggests that LXXLL peptides do not provide sufficient receptor binding affinity to permit a direct, binding assay.

In short, none of Heery's data suggest that the single peptides would be able to directly bind purified receptor proteins. Viewed through the eyes of one skilled in the art, Heery teaches away from an assay that relies on direct, in vitro, ligand-dependent LXXLL peptide binding to purified receptor, particularly one that requires lower, particularly sub-micromolar peptide concentrations.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application and any patent issuing therefrom.

Date: December 11, 2001

Steven L. McKnight